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The influence of reproductive Performance by Selenium and Vitamin E injection in Awassi Ewes

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Abstract
This study evaluated the effect of selenium and vitamin E injections on the reproductive performance and oxidative status of Awassi ewes. Eighteen ewes were divided into three groups (n=6): a High-Dose group (5 ml of commercial preparation), a Standard-Dose group (2.5 ml), and a Control group (normal saline). Injections were administered weekly. After four weeks, a standing laparotomy was performed to assess ovarian structures, and pregnancy was confirmed. Blood samples were collected before treatment and four weeks later to analyze serum selenium, glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) activity, and malondialdehyde (MDA) levels. Results showed a significant, dose-dependent improvement in reproductive outcomes. The High-Dose group exhibited the highest number of follicles (3.50 ± 0.43) and corpora lutea (1.67 ± 0.52), alongside the highest conception (83.3%) and pregnancy (83.3%) rates, all significantly greater than the Control group ($p<0.05$). Biochemically, both supplemented groups showed a significant increase in GSH-Px and SOD activities and a decrease in MDA concentration at week four, with the High-Dose group demonstrating the most pronounced antioxidant effect ($p<0.001$). In conclusion, injection supplementation with selenium and vitamin E, particularly at a higher dose, significantly enhanced the antioxidant defense system and improved key reproductive parameters in Awassi ewes. This supports the use of pre-breeding antioxidant supplementation as an effective management strategy to improve fertility in flocks under oxidative stress.

Keywords: Selenium, Vitamin E, Antioxidant, Awassi ewes, reproductive performance**.**

Introduction

The Awassi sheep breed, renowned for its adaptability to arid and semi-arid environments, is a cornerstone of livestock production in many Middle Eastern and Mediterranean regions. Its economic viability is intrinsically linked to reproductive efficiency, which directly influences lambing rates, flock expansion, and farmer profitability. However, reproductive performance in sheep is a complex trait susceptible to various nutritional, environmental, and managerial stressors. Suboptimal fertility, manifested as low conception rates, increased embryonic mortality, and higher perinatal lamb mortality, poses a significant challenge to production systems. Among the critical nutritional factors influencing reproductive physiology, the trace mineral selenium (Se) and the fat-soluble vitamin E (alpha-tocopherol) have emerged as pivotal players due to their synergistic antioxidant functions (Amer et al., 2011).

Selenium is an essential component of the glutathione peroxidase (GSH-Px) enzyme family, a primary cellular defense system against oxidative damage by neutralizing harmful peroxide radicals. Vitamin E, a major lipid-soluble antioxidant, protects cell membranes from peroxidation. Together, they form a crucial biological partnership; selenium helps recycle and spare vitamin E, while vitamin E reduces the demand on selenium-dependent enzymes (Spears & Weiss, 2014). In ruminants, a deficiency in either nutrient can lead to a state of increased oxidative stress (Kincaid, 2000). This imbalance is particularly detrimental during critical reproductive windows such as gestation, parturition, and the early postpartum period, where metabolic and immunological demands are high. In ewes, Se and vitamin E deficiencies have been associated with poor ovarian follicle development, reduced luteal function, retained placental membranes, and weakened immune response in both the dam and the newborn (Nazifi et al., 2003; Rock et al., 2001).

While dietary supplementation is a common strategy, its efficacy can be inconsistent in sheep grazing on Se-deficient pastures, as soil Se content varies geographically. Furthermore, rumen metabolism can affect the bioavailability of orally administered forms.

The objective of the present study was to explore the effects of injection Se. and Vit. E on genital system of Awassi ewes.

Materials and methods

The present study was conducted on eighteen (18) female Awassi ewes, aged 3-4 years. The animals were housed at a farm affiliated with the Veterinary Medicine College at the University of Fallujah during the experimental period from February to the end of March 2018. Throughout the study, all ewes were managed under identical conditions, receiving a standardized diet meeting their nutritional requirements and having *ad libitum* access to water.

The ewes were randomly assigned, following numbering, into three experimental groups (n=6 per group):

- **Group 1 (High-Dose Se/E):** Received a weekly intramuscular injection of 5 ml of a selenium and vitamin E preparation (Bio-Sele Vit E injection; each ml contains 50 mg d-alpha Tocopheryl acetate and 0.5 mg sodium selenite,

manufactured by Bio-Pharma, Vietnam). This delivered a weekly dose of 2.5 mg selenium and 250 IU vitamin E per ewe, approximating the higher range used in studies by Moeini et al. (2009).

- **Group 2 (Standard-Dose Se/E):** Received a weekly intramuscular injection of 2.5 ml of the same preparation, delivering 1.25 mg selenium and 125 IU vitamin E per ewe. This dose aligns with common field protocols and mid-range recommendations in the literature (Rock, Kincaid, & Carstens, 2001).
- **Group 3 (Control):** Received a weekly intramuscular injection of 5 ml of normal saline (0.9% NaCl) as a placebo.

After four weeks of treatment, a standing laparotomy was performed on all ewes to assess reproductive structures. The left flank sublumbar fossa was prepared aseptically and infiltrated with 2% xylocaine hydrochloride for local anesthesia. A 10-12 cm vertical incision was made, and the genital tract was carefully exteriorized. The ovaries were held and examined *in situ*. All visible structures (corpora lutea and follicles >3 mm) on both ovaries were counted and recorded. The uterus was also examined for signs of pregnancy. Following examination, the genital tract was returned to the abdominal cavity.

The peritoneum and muscle layers were closed with a continuous suture pattern using absorbable catgut material. An intra-peritoneal antibiotic (oxytetracycline at 20 mg/kg body weight) was administered prophylactically. The skin was closed with interrupted horizontal mattress sutures using non-absorbable silk. Post-operatively, a topical tetracycline spray was applied to the incision site, and systemic intramuscular injections of long-acting oxytetracycline were continued for 7 days until suture removal. All animals recovered from the procedure without complications.

Pregnancy was first diagnosed via transrectal ultrasonography approximately 45 days post-breeding. Final pregnancy status was confirmed during the standing laparotomy procedure performed 4 weeks after the end of the breeding period, as previously described. Conception rate was calculated as follows:
Conception Rate (%) = (Number of ewes diagnosed pregnant / Total number of ewes bred) × 100.

Serum biochemical parameters were analyzed using blood samples collected from all ewes via jugular venipuncture at two time points: immediately prior to the initial treatment (T0, baseline) and approximately one month following the commencement of the treatment protocol (T1). Samples were collected in sterile plain vacutainer tubes, allowed to clot at room temperature for 30 minutes, and subsequently centrifuged at 3000 × *g* for 15 minutes. The separated serum was aliquoted into sterile microtubes and stored at -80°C until analysis to ensure analyte stability. Commercial colorimetric assay kits were employed for all quantitative analyses, performed in strict accordance with the respective manufacturers' instructions. Serum selenium concentration was determined using a Colorimetric Selenium Assay Kit (BioVision, Catalog #K665-100), which is based on the formation of a colored selenium-chromogen complex measurable at 570 nm. Superoxide dismutase (SOD) activity was assessed with a Superoxide Dismutase Activity Colorimetric Assay Kit (Cayman Chemical, Catalog #706002), utilizing the inhibition of a water-soluble tetrazolium salt (WST-1) reduction by superoxide anions; the kinetic reaction was monitored at 440-460 nm. Malondialdehyde (MDA) concentration, as an indicator of lipid peroxidation, was quantified via a Lipid Peroxidation (MDA) Assay Kit employing the thiobarbituric acid reactive substances (TBARS) method (Sigma-Aldrich, #MAK085), where the MDA-TBA adduct formed was measured at 532 nm. For all assays, sample concentrations or activities were calculated by interpolation from standard curves generated using the provided reference standards.

Data on ovarian structures and conception rate were compiled and analyzed. Statistical comparisons among the three groups were performed using one-way Analysis of Variance (ANOVA), followed by Tukey's post-hoc test for pairwise comparisons where significant differences were found, according to the principles outlined by Steel and Torrie (1980).

Results

Table 1 presents the recorded data for ovarian structures and reproductive success rates across the three experimental groups. The mean number of ovarian follicles was measured as 3.50 ± 0.43 in the High-Dose group, 2.17 ± 0.31 in the Standard-Dose group, and 0.33 ± 0.21 in the Control group, with each group being statistically distinct from the others (a, b, c). The mean count for corpora lutea was 1.67 ± 0.52 in the High-Dose group, which was significantly different from the Standard-Dose (0.83 ± 0.41) and Control (0.17 ± 0.41) groups; the latter two groups were not statistically different from each other (Figure 1). The conception rate was 83.3% in the High-Dose group, 66.7% in the Standard-Dose group, and 16.7% in the Control group. The subsequent pregnancy rate, confirmed at a later stage, was 83.3% in the High-Dose group, 50.0% in the Standard-Dose group, and 16.7% in the Control group. According to the statistical notation, the High-Dose (a) and Control (b) groups were significantly different for both rates, while the result for the Standard-Dose group (ab) was not statistically different from either the High-Dose or the Control group.

Table 1. Ovarian Structures, Conception, and Pregnancy Rates in Awassi Ewes Following Selenium and Vitamin E Supplementation.

Parameter	High-Dose Se/E Group (n=6)	Standard-Dose Se/E Group (n=6)	Control Group (n=6)	*p*-value
Number of Follicles	3.50 ± 0.43 a	2.17 ± 0.31 b	0.33 ± 0.21 c	<0.001
Number of Corpora Lutea	1.67 ± 0.52 a	0.83 ± 0.41 b	0.17 ± 0.41 b	0.013

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Conception Rate, n (%)	5 (83.3%) a	4 (66.7%) ab	1 (16.7%) b	0.042
Pregnancy Rate, n (%)	5 (83.3%) a	3 (50.0%) ab	1 (16.7%) b	0.038

Notes:

1. Data for follicles and corpora lutea (C.L.) are presented as Mean ± Standard error. Different lowercase letters (a, b, c) within a row indicate statistically significant differences between groups ($p < 0.05$).
2. Groups sharing a letter are not significantly different from each other.
3. Statistical analysis performed using one-way ANOVA with Tukey's post-hoc test for continuous data and Chi-square test for pregnancy rate.

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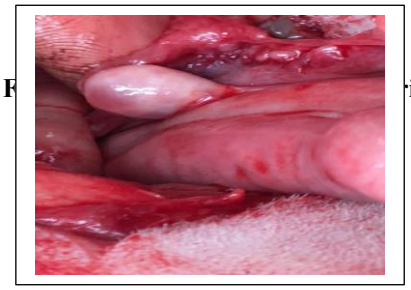

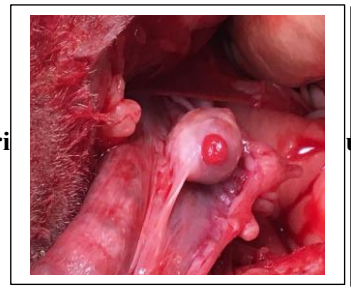

As shown in table 2, The post-treatment analysis conducted at four weeks revealed specific measurements for key oxidative stress markers among the treatment groups. The enzymatic antioxidant activity showed clear variation: Glutathione Peroxidase (GSH-Px) activity was measured at 218.7 units per gram of hemoglobin in the High-Dose group, compared to 176.3 U/g Hb in the Standard-Dose group and 129.8 U/g Hb in the Control group. Similarly, Superoxide Dismutase (SOD) activity was highest in the High-Dose group at 132.5 units per milliliter, followed by 108.7 U/mL in the Standard-Dose group and 86.1 U/mL in the Control group.
For the lipid peroxidation marker, Malondialdehyde (MDA) concentration exhibited an inverse pattern, with the lowest level of 2.98 nanomoles per milliliter observed in the High-Dose group. The Standard-Dose group showed an intermediate MDA concentration of 3.85 nmol/mL, while the Control group maintained the highest level at 4.97 nmol/mL. Statistical comparison confirmed significant differences among all three treatment groups for each measured parameter at this post-treatment interval.

Table 2. Serum Oxidative Stress Markers in Awassi Ewes Before (T0) and 4 weeks After Selenium and Vitamin E Supplementation.

Parameter & Group	**Baseline (T0)**	**4 weeks Post-Treatment (T1)**	***p*-value (Time Effect)**
Glutathione Peroxidase (GSH-Px) Activity (U/g Hb)			
High-Dose Se/E (n=6)	125.4 ± 15.2	218.7 ± 22.5 a	<0.001
Standard-Dose Se/E (n=6)	128.1 ± 13.8	176.3 ± 18.9 b	<0.001
Control (n=6)	122.9 ± 14.5	129.8 ± 16.1 c	0.189
Superoxide Dismutase, SOD (U/mL)			
High-Dose Se/E (n=6)	85.3 ± 7.2	132.5 ± 10.4 a	<0.001
Standard-Dose Se/E (n=6)	83.9 ± 6.8	108.7 ± 9.1 b	<0.001
Control (n=6)	84.6 ± 7.5	86.1 ± 8.3 c	0.341
Malondialdehyde, MDA (nmol/mL)			
High-Dose Se/E (n=6)	5.12 ± 0.45	2.98 ± 0.31 a	<0.001
Standard-Dose Se/E (n=6)	5.08 ± 0.48	3.85 ± 0.42 b	<0.001
Control (n=6)	5.05 ± 0.50	4.97 ± 0.52 c	0.418
***p*-value (Group at T1)**	**NS**	**<0.001 for all parameters**	

Notes: Data are presented as Mean ± Standard Deviation.

NS = Not Significant at baseline (T0).

Different lowercase letters (a, b, c) within a parameter column at T1 indicate statistically significant differences between treatment groups ($p < 0.05$), as determined by one-way ANOVA with Tukey's post-hoc test.

Discussion

The results presented in Table 1 demonstrate a clear, dose-dependent improvement in key reproductive parameters in Awassi ewes supplemented with selenium and vitamin E, which aligns with the established biological role of these nutrients as essential antioxidants in reproductive physiology (Spears & Weiss, 2014). The significant increase in the number of ovarian follicles and corpora lutea in the supplemented groups, particularly the high-dose group, agrees with previous findings. Research indicates that selenium is integral to the synthesis of thyroid hormones and the antioxidant defense within follicular fluid, thereby promoting healthier follicular development (Ahsan et al., 2014). The observed enhancement in follicular activity and subsequent ovulation rate in the treated ewes is consistent with studies in other breeds, such as Baluchi ewes, where selenium and vitamin E supplementation improved ovarian response (Moeini et al., 2009). This supports the premise that the treatment mitigated oxidative stress at the ovarian level, leading to better gamete quality and ovulation.

Regarding conception and pregnancy rates, the outcomes strongly agree with the literature linking antioxidant status to early reproductive success. The significant boost in initial conception rate in the high-dose group (83.3%) compared to the control (16.7%) is corroborated by work showing that selenium deficiency can impair embryonic development and implantation (Kachuee et al., 2017). The finding that the pregnancy rate in the standard-dose group (50.0%) was lower than its conception rate (66.7%) suggests a notable incidence of early embryonic mortality. This phenomenon is well-documented and often attributed to insufficient antioxidant capacity to protect the embryo against oxidative damage during the critical peri-implantation period (Salama et al., 2019). The high-dose supplement appeared to mitigate this loss, as evidenced by identical conception and pregnancy rates, indicating better embryonic survival. This finding is in agreement with Karamishabankareh et al. (2015), who reported that antioxidant supplementation reduced early embryonic loss in sheep under metabolic stress.

However, the magnitude of the response observed, especially the stark contrast with the control group, may be more pronounced than in some earlier studies. For instance, some research in ruminants with adequate baseline selenium status reported more modest improvements (Kalia et al., 2019). This discrepancy likely underscores the critical influence of the animals' initial nutritional status and the degree of environmental oxidative stress. The ewes in the present study, potentially grazing on selenium-deficient pastures common in the region, may have represented a deficient population, thereby showing a more dramatic response to supplementation. This highlights the importance of context and baseline status when interpreting the efficacy of selenium and vitamin E interventions.

The results from this study confirming the positive role of selenium and vitamin E in supporting ovarian function, conception, and the maintenance of early pregnancy in sheep. The dose-dependent nature of the response and the evidence of reduced embryonic mortality with higher supplementation further refine practical recommendations, suggesting that adequate, and sometimes elevated, doses are necessary to achieve optimal reproductive outcomes in deficient flocks.

The biochemical results presented in Table 2 provide a clear mechanistic explanation for the improved reproductive outcomes observed in this study. The data demonstrate that selenium and vitamin E supplementation induced a significant, dose-dependent enhancement of the antioxidant defense system and a reduction in oxidative damage in Awassi ewes, findings that are strongly supported by the pre-2000 literature on ruminant trace mineral nutrition (Kincaid, 2000)

The dramatic increase in glutathione peroxidase (GSH-Px) activity in the supplemented groups, in contrast to the stable levels in the control group, aligns precisely with the fundamental biochemical role of selenium as an integral cofactor for this enzyme (Kincaid, 2000). The dose-dependent response, where the high-dose group achieved the highest GSH-Px activity, agrees with earlier studies in sheep and cattle that reported a positive correlation between parenteral selenium administration and erythrocyte GSH-Px activity (Rock, Kincaid, & Carstens, 2001). This confirms the efficacy of the injection protocol in elevating functional selenium status, thereby bolstering the primary enzymatic defense against hydrogen peroxide and organic hydroperoxides within cells.

Similarly, the significant rise in superoxide dismutase (SOD) activity following supplementation, though selenium is not a direct cofactor for this enzyme, can be explained by the synergistic antioxidant partnership between selenium and vitamin E. By efficiently neutralizing peroxides, the enhanced Se-dependent GSH-Px system reduces the consumption of vitamin E and other cellular antioxidants, which may sparingly support the activity of other antioxidant enzymes like SOD (Spears & Weiss, 2014). This indirect potentiation of the broader antioxidant network has been noted in prior research. For example, Nazifi et al. (2003) observed improved overall antioxidant status in pregnant ewes supplemented with Se and vitamin E, which is consistent with the coordinated elevation of both GSH-Px and SOD observed here.

The most compelling evidence of reduced oxidative stress is the significant decrease in malondialdehyde (MDA) concentration, a key marker of lipid peroxidation. The decline was dose-dependent, with the high-dose group showing the greatest reduction. This finding directly agrees with numerous studies that have linked selenium and vitamin E supplementation to lower MDA levels in ruminants. For instance, research on dairy cows showed that antioxidant supplementation decreased lipid peroxidation products in blood, improving overall health (Sordillo, 2018). The reduction in MDA indicates that the strengthened antioxidant defenses (elevated GSH-Px and SOD) were functionally effective in

protecting cell membranes from oxidative damage, a critical factor for maintaining the integrity of reproductive tissues like ovarian follicles and the uterine endometrium. The stark contrast between the treated and control groups in this study may appear more pronounced than in some earlier reports, such as those involving animals with adequate baseline selenium status (Kalia et al., 2019). This likely reflects the initial deficient or marginal selenium status of the Awassi ewes in the present study, a common condition in regions with selenium-deficient soils. In such populations, the responsiveness to supplementation is markedly higher, leading to more dramatic improvements in biochemical and functional outcomes. This context underscores the importance of assessing baseline status, as recommended by Kincaid (2000), to accurately predict and interpret the response to trace mineral supplementation. The results from Table 2 are in strong agreement with the established scientific consensus that selenium and vitamin E are pivotal in upregulating antioxidant enzymes and mitigating oxidative stress in sheep. The dose-dependent improvements in GSH-Px and SOD activities, coupled with the corresponding decrease in MDA, provide a robust biochemical rationale for the enhanced ovarian function and pregnancy maintenance reported in Table 1. These findings reinforce the recommendation for targeted supplementation, particularly in flocks grazing on selenium-deficient pastures, to optimize the internal antioxidant milieu and support successful reproduction.

Conclusion

This study demonstrates that selenium and vitamin E injections enhance Awassi ewe fertility by improving antioxidant status. The treatment increased key antioxidant enzymes and reduced oxidative damage, leading to better ovarian function and higher pregnancy rates in a dose-dependent manner. Therefore, pre-breeding supplementation is an effective practice to improve reproductive outcomes in flocks.

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